Sex determination: ways to evolve a hermaphrodite.

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Sex determination: Ways to evolve a hermaphrodite

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Summary

Most species of the nematode genus *Caenorhabditis* reproduce through males and females. *C. elegans* and *C. briggsae*, however, produce self-fertile hermaphrodites instead of females. These transitions to hermaphroditism evolved convergently through distinct modifications of germline sex determination mechanisms.

In *C. elegans*, sex determination mechanisms have been studied in detail and involve a common molecular pathway in somatic and germline cells. Recent studies use this knowledge to ask how sex determination evolves among species closely related to *C. elegans* [1,2]. Of particular interest is to understand how this process was modified to produce alternative mating systems.

Convergent evolution of hermaphroditism in a female soma

The genus *Caenorhabditis* currently encompasses eleven species in culture [3], nine of which produce females and males, while two, *elegans* and *briggsae*, produce self-fertile hermaphrodites and males. Their phylogenetic relationships suggest that hermaphroditism evolved twice independently from an ancestral male-female mating system [3-5] (Figure 1). In both species, the hermaphrodite anatomically resembles a female, which undergoes spermatogenesis for a brief period prior to oogenesis (Figure 2A). The hermaphrodite can then self-fertilize using its own sperm (or mate with a male).

Sex determination in these nematodes is chromosomal: females and hermaphrodites carry two X chromosomes while males carry one (as a result of rare X meiotic non-disjunction, or of crosses between a male and a female/hermaphrodite). In males, the germline is exclusively male, in females exclusively female, while in hermaphrodites, the first gametes differentiate as sperm and the following as oocytes. Evolution to hermaphroditism thus occurs through the modulation of germline sex determination in XX animals.

Conserved determinants of germline sex

In *C. elegans*, a common pathway determines the sexual identity of soma and gametes (Figure 2B). In males, low X dosage results in the expression of HER-1, a secreted protein, which inhibits TRA-2, a Patched–like receptor. A signal transduction pathway through FEM-1, FEM-2 and FEM-3 then represses the TRA-1 (Gli/Ci-like) transcription factor. In the hermaphrodite germline, spermatogenesis is activated via a modification of this pathway at the level of *tra-2* mRNA. As in the male soma, *tra-2* repression then leads via the FEMs to TRA-1 inhibition. Downstream, the key germline-specific transcriptional target of TRA-1 is *fog-3*: inhibition of TRA-1 activates *fog-3* transcription and thereby spermatogenesis. The switch to oogenesis switch then operates at the level of *fem-3* translational repression [6].

In *C. briggsae* and *C. remanei*, the sex determination regulators are overall functionally conserved, despite rapid sequence evolution and several examples of protein co-evolution [1,7]. Concerning the germline, the role of *fog-3* appears conserved: RNAi experiments suggest that *fog-3* promotes spermatogenesis in males of all three species and in *elegans* and *briggsae* hermaphrodites. Moreover, *fog-3* is expressed in the germline of *elegans* and *briggsae* hermaphrodites, but not in that of *remanei* females [8]. Therefore, a common feature in the evolution of hermaphroditism of *elegans* and *briggsae* is *fog-3* activation in the germline of XX animals.

Moving up the pathway, *fog-3* expression is regulated in *C. elegans* by TRA-1, and TRA-1 binding sites in *fog-3* regulatory sequences are conserved in *briggsae* and *remanei* [1,8]. However, further up the pathway, modulation of germline sex differs between *elegans* and *briggsae*.

Spermatogenesis without *fem-2* and *fem-3* in *C. briggsae*

In *C. elegans*, hermaphrodite spermatogenesis requires the inhibitory action of FEM-1, FEM-2 and FEM-3 on TRA-1 (Figure 2B): mutants in either of these *fem* genes are transformed into females (soma and germline). In *C. briggsae*, their RNAi inactivation did not produce this phenotype [9,10], but the poor efficiency of RNAi required a better test. Recent work by Hill et al. [2] beautifully answers this problem by 1) isolating deletion alleles of *Cb-fem-2* and *fem-3* and 2) screening for *Cb-tra-2* suppressors. This is the first published study applying targeted gene deletion and systematic mutagenesis in *C. briggsae*, a very promising result for future work using this species.

The *C. briggsae fem-2* and *fem-3* deletions were screened by PCR of EMS-mutagenized worm pools. The resulting *Cb-fem-2* and *fem-3* mutants (and double mutants between them) develop into self-fertile hermaphrodites– no matter whether the animals bear one or two X chromosomes [2]. These genes are thus required for somatic male identity as in *C. elegans*; however, for hermaphrodite spermatogenesis, they are essential in *C. elegans*, yet dispensable in *C. briggsae*.

FEM-2 and FEM-3 are required for signal transduction from TRA-2 to TRA-1. In both *elegans* and *briggsae*, *tra-2* inactivation transforms XX animals into males (soma and germline). In an extensive screen for *Cb-tra-2* suppressors, Hill et al. identified numerous mutations causing somatic feminization, including *Cb-fem-2* alleles. However, these mutants all developed into self-fertile hermaphrodites rather than females [2]. This result corroborates the fact that, unlike in *C. elegans*, hermaphrodite spermatogenesis in *C. briggsae* does not require the same *tra-2* downstream genes (such as *fem-1/2/3*) as in the male soma.

fog-2* is unique to *C. elegans

Upstream of *tra-2* in *C. elegans*, the specific regulator of hermaphrodite spermatogenesis is *fog-2*, which together with *gld-1* represses *tra-2* mRNA (Figure 2B). *fog-2* is required for hermaphrodite spermatogenesis but, unlike *fog-3*, not for male spermatogenesis [6].

Most sex determination factors identified in *C. elegans* have orthologues in *C. briggsae* with one interesting exception: *fog-2*. This gene seems to have arisen in a gene family expansion in the evolutionary branch leading to *C. elegans*. In *C. briggsae*, *fog-2* is not only absent but *gld-1* plays an opposite role: its inactivation suppresses oogenesis in XX animals [1].

Convergent evolution through distinct molecular mechanisms

Taken together, these studies demonstrate that the convergent evolutionary transition to hermaphroditism in *C. briggsae* and *C. elegans* likely involved alternative modifications of the sex determination pathway.

In *C. elegans*, gene duplication and divergence leading to *fog-2* may have been a key factor in the evolution of hermaphroditism, transforming germline fate through *tra-2* mRNA repression and *fem-2/3* activity.

In *C. briggsae*, the control of hermaphrodite spermatogenesis ultimately occurs through *fog-3* regulation, but without a *fog-2* orthologue and without requiring *fem-2* and *fem-3*. *tra-2* 3'UTR regulation appears conserved in *C. briggsae* [11], although its role in hermaphrodite spermatogenesis was not tested. A direct action of TRA-2 on TRA-1 may regulate *fog-3* in *C. briggsae*, bypassing FEM-1/3. Interestingly, direct cross-regulations between *tra-1* and *tra-2* are known in *C. elegans* [6].

One step towards understanding the transition to hermaphroditism will be to characterize sex determination in closely related male-female species, such as *C. remanei*. It may also be interesting to compare sex determination in wild isolates of a given species. There is evidence of genetic variation in the timing of the sperm-to-oocyte switch that determines sperm and thus hermaphrodite self-progeny number [12-14]. This suggests an additional, quantitative modulation of the germline sex determination mechanisms within species.

Evolution of mating systems

The current evidence suggests that switching between alternative mating systems is easy in *Caenorhabditis* – mechanistically and evolutionarily. It may only take one or two mutations to shift between different reproductive modes, and these shifts seem largely limited to the germline with little pleiotropic effects. Moreover, mutation of different genes can cause transformation into the same reproductive phenotype. These factors may create the potential for rapid and frequent evolutionary transitions between mating systems in *Caenorhabditis* and other rhabditids.

Ultimately, what are the evolutionary causes favouring the adoption of a particular mating system and what are the consequences of its maintenance? Understanding the origin and maintenance of sexual reproduction (outcrossing) is a key topic in evolutionary biology, and *Caenorhabditi*s species may be particularly well-suited to address this problem. A selfing mode of reproduction has in principle an advantage over sexual reproduction (two-fold cost of sex). In addition, the ability of an individual to self-fertilize obviates the need for a mating partner. This may be advantageous to organisms that colonize ephemeral habitats where population densities fluctuate dramatically, as observed for *Caenorhabditis* species [3, 15]. On the other hand, selfing rapidly increases levels of homozygosity (inbreeding) and novel deleterious mutations cannot be purged through sexual recombination. Analysing the relative costs and benefits of selfing versus outcrossing – in the short and long term – are therefore crucial in explaining the evolution of alternative mating systems. The hermaphroditic *C. elegans* and *C. briggsae* are capable of producing functional males that allow outcrossing events. Several lines of evidence suggest that *C. elegans* and *C. briggsae* maintain a very low yet detectable outcrossing rate [15,16] (Cutter, Félix, Barrière & Charlesworth, submitted). Such partial outcrossing of a predominant selfer may combine the advantages of both selfing and outcrossing [17]. The further integration of evolutionary, ecological and developmental studies on different *Caenorhabditis* species presents a promising approach to clarify the proximate and ultimate causes of mating system evolution.

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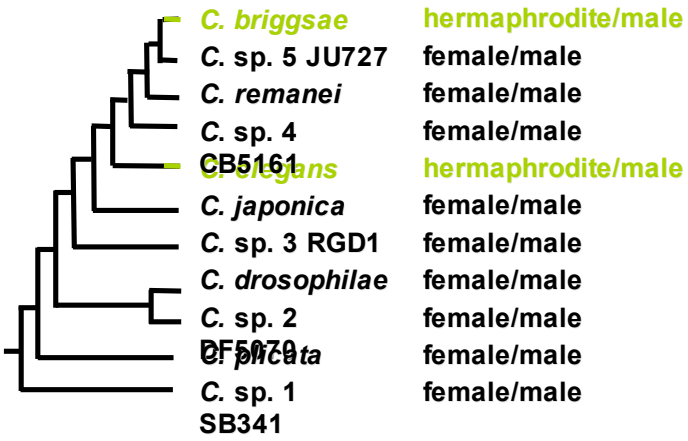

Figure 1. Convergent evolution of hermaphroditism in *C. elegans* and *C. briggsae*. Molecular phylogeny of the genus *Caenorhabditis*, after [3]. Hermaphroditism (green) appears to have evolved independently in *C. elegans* and *C. briggsae*.

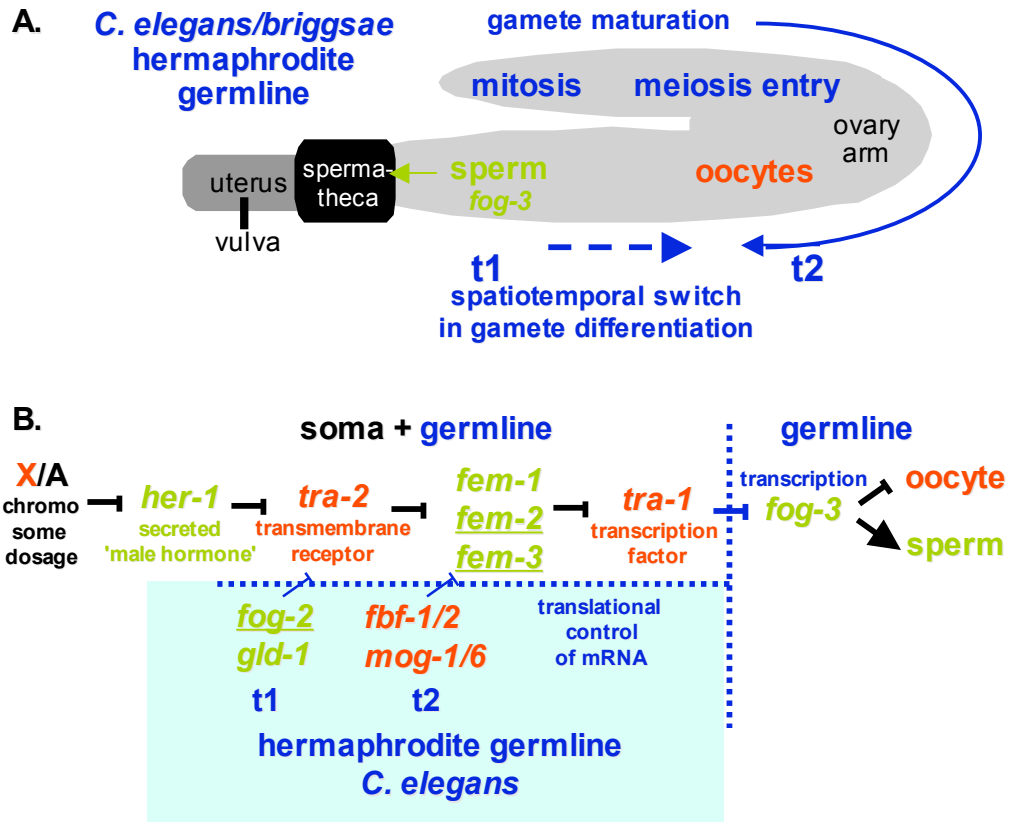

Figure 2. Germline sex determination in *C. elegans* versus *C. briggsae*.
A. Temporal switch in hermaphrodite germline differentiation. In both species, *fog-3* is required for spermatogenesis onset. Sperm cells develop at the proximal end of the arm and then move to the spermatheca. Oocytes mature from the distal end of the arm throughout adulthood. Only one of the two gonadal arms is represented. In case of outcrossing, male sperm enter through the vulva and likewise reach the spermatheca.
B. Genetic pathway underlying somatic and germline sex determination. Genes whose products are present or activated in male-fated cells are in green, those active in female-fated cells are in red. Germline-specific regulatory components are in blue. Genes that are underlined are absent in the *C. briggsae* genome (*fog-2*) or do not participate to the regulation of hermaphrodite spermatogenesis in this species (*fem-2* and *fem-3*). Genes are named after their mutant phenotype in *C. elegans*: Her (hermaphrodization of XO animals), Tra (transformation of XX animals to males), Fem (feminization of XX and XO animals), Fog (feminization of the germline), Mog (masculinization of the germline). After [2].